Cross-calibration of the racemization rates of leucine and phenylalanine and epimerization rates of isoleucine between ostracodes and gastropods over the Pleistocene in southern Spain

J.E. Ortiz*, T. Torres, F.J. Llamas

Laboratory of Biomolecular Stratigraphy, E.T.S.I. Minas de Madrid, C/ Ríos Rosas 21, Madrid 28003, Spain

Abstract

Cross-calibration of the racemization rates of leucine and phenylalanine and the epimerization rates of isoleucine between Middle and Lower Pleistocene ostracodes and gastropods from southern Spain is presented. Using these two relationships, along with previously-calculated age estimation algorithms, it is possible to estimate the age of samples from southern and central Spain because of the shared thermal history of these regions. They can also be used to establish the aminostratigraphy of Quaternary deposits from the areas. However, for D/L ratios below those in the ostracodes and gastropods of the Cortijo del Negro-1 site, the cross-calibration equations may not be satisfactorily applied. The racemization/epimerization ratios in ostracode shells can be measured precisely because of the excellent preservation of amino acids within an valves and an abundance that makes the standard error or variance smaller. It has been observed that in modern samples the racemization in ostracodes is slower than in gastropods but the D/L ratios become similar in older samples.

1. Introduction

In recent years, the amino acid racemization method has become one of the most widely used geochronological tools for dating both continental and marine Quaternary deposits. Likewise, it can be used for stratigraphical correlation purposes.

Living beings contain only L-amino acids which gradually racemize to D-amino acids after death. Thus, the D/L ratio increases with time after death until it is equal to 1, that is, when equilibrium is reached. Amino acids with two asymmetrical carbon atoms (such as isoleucine) undergo epimerization, which is the transformation of an L-diastereomer into another D-diastereomer. Each amino acid has a unique racemization or epimerization rate.

Because samples usually come from strata deposited under a diversity of palaeoecological niches, it is necessary to analyze samples of a wide variety of species, genera or even orders to establish the aminostratigraphical arrangement of the studied sites, from D/L ratios alone, or to calculate ages from previously defined mathematical algorithms. However, the racemization rate varies depending on the fossil genus and, obviously, taxon; Murray-Wallace (1995) estimated that the variation of the genus effect was 30%. According to King and Neville (1977) and Miller et al. (1983) the rate of isoleucine epimerization in foraminifera is strongly genus-dependent in both planktonic and benthic species. Miller et al. (1983) also noticed significant divergence in epimerization values between pelecypods and benthic foraminifera. Hearty et al. (1986) and Hearty (1987)

found differences in the D-alloisoleucine/L-isoleucine (D-aIle/L-Ile) values between *Glycymeris* sp. and *Arca* sp. from the same strata along Mediterranean coasts. Likewise, Torres et al. (2000) reported differences in the D/L ratios of aspartic acid (Asp), glutamic acid (Glu), isoleucine and leucine (Leu) between shells of diverse pelecypoda genera from the east coast of Spain. Finally, Goodfriend and Stanley (1996) estimated the age calculation equation for the D/L Asp ratio in a pelecypod (*Corbicula* sp.) from the Nile delta from comparison of the D/L Asp racemization rates with those for another pelecypod (*Cerastoderma* sp.).

A wide group of materials is available for dating with this method but, in spite of the abundance of ostracodes, their prevalence in most lacustrine environments and their convenience to work with, there are only a few studies based on their D/L ratios (McCoy, 1988; Torres et al., 1995; Oviatt et al., 1999; Ortiz, 2000; Ortiz et al., 2000; Kaufman, 2000).

According to our experience (Ortiz, 2000) ostracodes have two main characteristics that make them particularly useful for amino acid racemization/epimerization dating:

1. The excellent preservation of amino acids in their valves allows analysis of a small sample size (10–20 mg) by gas chromatography (GC), much less than for other organisms, e.g. molluscs (80 mg). Using reversed phase high performance liquid chromatography (HPLC), it is possible to analyze even a single ostracode valve (cf. Kaufman, 2000).
2. In a single GC analysis, there are typically between 1500 and 2000 ostracode valves, so the standard error or variance is low because the sample is statistically significant.

Furthermore, in most cases, ostracodes are abundant and the only fauna present in outcrops, or along both stratigraphic sections and drill cores, so gastropods or bivalves cannot be used to obtain an entire and accurate amino acid chronology.

Until now we have developed age calculation algorithms to estimate the age of Pleistocene deposits using racemization/epimerization ratios of different amino acids (leucine, isoleucine, phenylalanine, aspartic acid and glutamic acid) of fossil gastropods (cf. Torres et al., 1997; Ortiz, 2000). For this purpose deposits dated from absolute methods were used. However, palaeoenvironmental characteristics have resulted in not all the gastropod-bearing strata being used in the calculation of these algorithms. When ostracodes were present, only in a few cases were we able to collect the necessary ca. 1500 valves.

Because of these reasons and the particularly good characteristics for ostracodes it is obvious that it would be useful to determine the equations that relate their amino acid racemization and epimerization ratios to those of other groups of fossils such as gastropods in order to obtain or improve either the aminostratigraphy or the aminochronology of an area.

We have chosen to work with gastropods and ostracodes from the Cúllar-Baza Basin, where they are omnipresent. This basin is a "basin-and-range" zone located (Fig. 1) in the southeast of the Iberian Peninsula, in the central part of the Betic Range and is filled by alluvial and lacustrine-palustrine deposits of Pliocene and Pleistocene age. It is one of the few areas of Europe and the only one in Spain where almost continuous sedimentation took place over nearly the whole of the Quaternary. A detailed stratigraphical and paleontological description is given in Ortiz (2000).

Samples were recovered from five paleontological localities (Cortijo del Negro-1, Fuente Amarga-1, Cúllar-Baza-1, Cortes de Baza-182 and Venta Micena-1), developed under palustrine conditions, whose geographical and biostratigraphical details appear in Table 1.

2. Material and methods

The ostracodes from the Cortijo del Negro-1, Fuente Amarga-1, Cúllar-Baza-1 and Cortes de Baza-182 paleontological sites were classified as *Cyprideis torosa* (Jones), while in Venta Micena-1 they belong to two genera: *Ilyocypris* and *Cyprideis* because, due to the numbers necessary for GC analysis it was impossible at this site to separate a sample comprising only a single genus. In spite of the racemization process being genus-dependent, these two different ostracode genera were employed to calculate the cross-calibration racemization and epimerization rates. In fact, in previous studies (McCoy, 1988; Oviatt et al., 1999; Kaufman, 2000) only slight differences between D/L ratios from different phylogenetic ostracode groups (Superfamilies Cypridacea and Cytheracea) were reported. In the present work, the ostracodes from Venta Micena-1 belong to either one of the two Superfamilies: *Ilyocypris* to Superfamily Cypridacea and *Cyprideis* to Superfamily Cytheracea.

Because Torres et al. (1995,1997) found only small differences between racemization ratios in land and aquatic snail samples, we decided to combine them, describing them as Gastropoda but including *Helix* (the most abundant), *Planorbis*, *Lymnaea*, *Radix*, *Bithynia* genera representatives.

Once the samples were recovered, they were sieved under running water and dried at room temperature. They were then studied under a binocular "Wild" microscope to determine the lithology and faunal assemblages. Ostracodes were carefully sonicated and cleaned with water to remove sediment which might

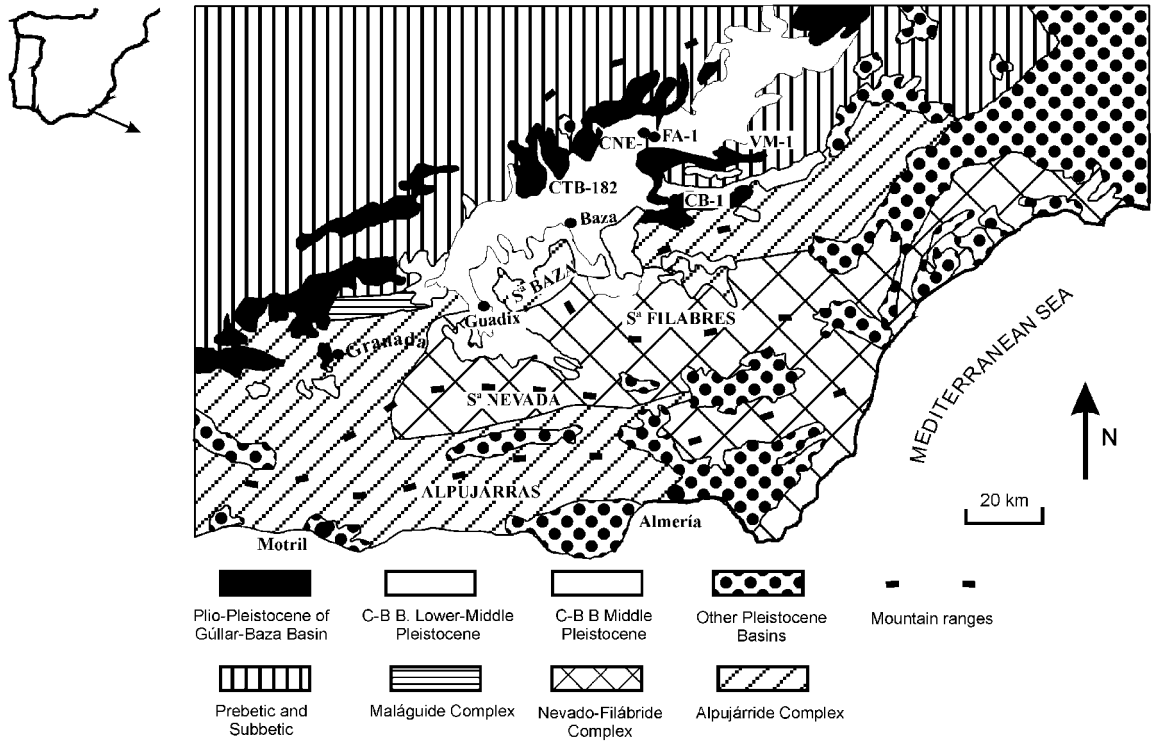

Fig. 1. Geographical setting of the Cúllar-Baza Basin and location of the palaeontological sites: Venta Micena-1 (VM-1), Cortes de Baza-182 (CTB-182), Cúllar-Baza-1 (CB-1), Fuente Amarga-1 (FA-1) and Cerro del Negro-1 (CNE-1).

Table 1
Geographical location and geological age of Cúllar-Baza basin paleontological sites

Locality	Latitude	Longitude	Elevation (m)	Geological age
Venta Micena (VM-1)	37°44′8″	2°24′27″	960	(Upper Villafranchian) Lower Pleistocene Martínez Navarro (1992), Sesé (1994); Torres et al. (1997)
Cortes de Baza-182 (CTB-182)	37°39′7′	2°45′2″	760	Lower Pleistocene Oms et al. (1994)
Cúllar Baza-1 (CB-1)	37°34′10″	2°33′50″	940	Galerian (Middle Pleistocene) Sesé (1994), Torres et al. (1997)
Fuente Amarga-1 (FA-1)	37°46′7″	2°35′12″	880	Middle Pleistocene Torres et al. (1995)
Cortijo del Negro-1 (CNE-1)	37°46′27″	2°36′21″	905	Not previously dated

have been contained in their valves. Mollusc shells were also washed with running water and cleaned by extensive sonication. Afterwards, we isolated amounts of 80 mg of gastropods and 15–20 mg of ostracodes.

The sample preparation protocol is described in Goodfriend (1991) and Goodfriend and Meyer (1991) and involves:

1. Hydrolysis which was performed under a N_2 atmosphere in a mixture of 12 N HCl and shell carbonate (2.9 μl/mg) and 6 N hydrochloric acid (100 μl) for 20 h at 100 °C; samples were then desalted in HF and the resultant supernatant frozen and dried under vacuum.
2. Derivatization: amino acids were derivatized in a two step process, involving first esterification with 250 μl of 3 M thionyl chloride in isopropanol for 1 h at 100 °C under N_2; the samples were dried and acylated by reaction with 150 μl of trifluoroacetic acid anhydride (25% in dichloromethane) for 5 min at 100 °C. Excess derivative and solvent were evaporated under a

gentle flow of nitrogen. The sample was taken up in 125 μl of *n*-hexane which was vortexed and the solvent was reduced in a stream of N_2 to a final volume of 15–25 μl.

Aliquots (1–4 μl) were injected into a Hewlett-Packard 5890 gas chromatograph. The injection port was kept at 215 °C and set for splitless mode for the first 75 s, at the beginning of which the sample was injected, and later set to split mode. We used helium as the carrier gas, at a column head pressure of 5.8 psi, and a Chirasil-L-Val fused silica column (25 m×0.38 mm) from Chrompack. The gradients used were as follows: 50 °C (1 min), heating at 40 °C/min to 115 °C, 12 min at 115 °C, 3 °C/min to 190 °C, 10 min at 190 °C. The detector was an NPD set at 300 °C. Integration of the peak areas was carried out using the HP PEAK96 integration program. As a laboratory routine, D/L-alanine, D/L-valine, D-alloisoleucine/L-isoleucine, D/L-proline, D/L-aspartic acid, D/L-leucine, D/L-phenylalanine and D/L-glutamic acid peaks were identified.

3. Results and discussion

The results for the mean values of the D/L ratios of five different amino acids in ostracodes and gastropods (Table 2) were plotted on XY graphs. Mean D/L ratios for each amino acid were regressed linearly and logarithmically in order to select the best trend. With these correlations, "equivalent ratios" can be obtained from different D/L ratios of amino acids analyzed in ostracodes, which are defined as the D/L ratios that a gastropod from a similar horizon would have. The results of the attempted linear approach are:

$$\text{D-aIle/L-Ile}_{\text{equivalent}} = 0.39133 + 0.67542 \times \text{D-aIle/L-Ile}_{\text{ostracodes}};\quad r = 0.983,\ P = 0.003$$

$$\text{D/L LEU}_{\text{equivalent}} = 0.42581 + 0.50249 \times \text{D/L LEU}_{\text{ostracodes}};\quad r = 0.957,\ P = 0.011$$

$$\text{D/L ASP}_{\text{equivalent}} = 0.30776 + 0.72441 \times \text{D/L ASP}_{\text{ostracodes}};\quad r = 0.749,\ P = 0.145$$

$$\text{D/L PHE}_{\text{equivalent}} = 0.54654 + 0.38697 \times \text{D/L PHE}_{\text{ostracodes}};\quad r = 0.957,\ P = 0.011$$

$$\text{D/L GLU}_{\text{equivalent}} = 0.38005 + 0.51751 \times \text{D/L GLU}_{\text{ostracodes}};\quad r = 0.840,\ P = 0.075$$

where r is the correlation coefficient and P is the significant level.

The results of the logarithmic approach are:

$$\text{D-aIle/L-Ile}_{\text{equivalent}} = 1.0716 + 0.40618 \times \text{Ln(D-aIle/L-Ile}_{\text{ostracodes}});\quad r = 0.997,\ P = 0.000$$

$$\text{D/L LEU}_{\text{equivalent}} = 0.84298 + 0.1990 \times \text{Ln(D/L LEU}_{\text{ostracodes}});\quad r = 0.869,\ P = 0.056$$

$$\text{D/L ASP}_{\text{equivalent}} = 0.98219 + 0.45856 \times \text{Ln(D/L ASP}_{\text{ostracodes}});\quad r = 0.759,\ P = 0.137$$

Table 2
D-aIle/L-Ile and D/L mean values for amino acids in gastropoda and ostracodes from Venta Micena-1 (VM-1), Cortes de Baza-1 (CTB-1), Cúllar-Baza-1 (CB-1), Fuente Amarga-1 (FA-1) and Cortijo del Negro-1 (CNE-1) sites

Sample	Material	*n*	D-aIle/L-Ile		D/L Leu		D/L Asp		D/L Phe		D/L Glu	
			Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
VM-1	Gastropoda	7	1.160	0.031	0.893	0.017	0.922	0.012	0.896	0.013	0.835	0.033
	Ostracoda	3	1.156	0.057	0.848	0.004	0.756	0.011	0.850	0.062	0.720	0.010
CTB-182	Gastropoda	3	1.045	0.032	0.760	0.011	0.755	0.009	0.818	0.029	0.690	0.031
	Ostracoda	3	0.998	0.019	0.710	0.026	0.742	0.035	0.804	0.005	0.750	0.083
CB-1	Gastropoda	12	0.828	0.030	0.639	0.054	0.809	0.016	0.799	0.027	0.633	0.019
	Ostracoda	8	0.559	0.040	0.479	0.065	0.575	0.005	0.485	0.074	0.372	0.010
FA-1	Gastropoda	13	0.713	0.056	0.577	0.052	0.711	0.023	0.711	0.043	0.531	0.038
	Ostracoda	1	0.419	0.000	0.376	0.000	0.587	0.000	0.373	0.000	0.364	0.000
CNE-1	Gastropoda	2	0.480	0.105	0.563	0.040	0.624	0.016	0.616	0.026	0.534	0.015
	Ostracoda	5	0.228	0.019	0.181	0.021	0.494	0.010	0.240	0.024	0.349	0.005

n, Number of analysis; S.D.; standard deviation.

$$\text{D/L PHE}_{\text{equivalent}} = 0.89643 + 0.19391 \times \text{Ln(D/L PHE}_{\text{ostracodes}}); \quad r = 0.972, P = 0.006$$

$$\text{D/L GLU}_{\text{equivalent}} = 0.84675 + 0.27574 \times \text{Ln(D/L GLU}_{\text{ostracodes}}); \quad r = 0.850, P = 0.068$$

Only the linear correlations obtained for isoleucine, leucine and phenylalanine were significant ($P<0.05$), with high correlation coefficients. Based on the correlation coefficients, the logarithmic trend is the best fit for isoleucine (Fig. 2) and phenylalanine (Fig. 3) whereas for leucine (Fig. 4) the best fit is a linear trend. These results cannot be extrapolated to lower gastropod and ostracode racemization ratios than those obtained in the Cortijo del Negro-1 sample (see Table 2), in our opinion, because the racemization/epimerization is a non-linear process (Goodfriend, 1991) in which the racemization rate eventually decreases with time. In fact, some authors have found different behaviour beyond D/L ratios of 0.3 (Masters and Bada, 1977; Kriausakul and Mitterer, 1980) or 0.5 (Wehmiller and Hare, 1971; Bada and Schroeder, 1972), depending on the material.

The results for aspartic acid and glutamic acid are not satisfactory. Among the five amino acids used, aspartic acid and glutamic acid racemize faster than phenylalanine, leucine and isoleucine. In our opinion this could explain the poor correlation between aspartic acid and glutamic acid D/L ratios of ostracodes and gastropods. This is confirmed by the results obtained by Torres et al.

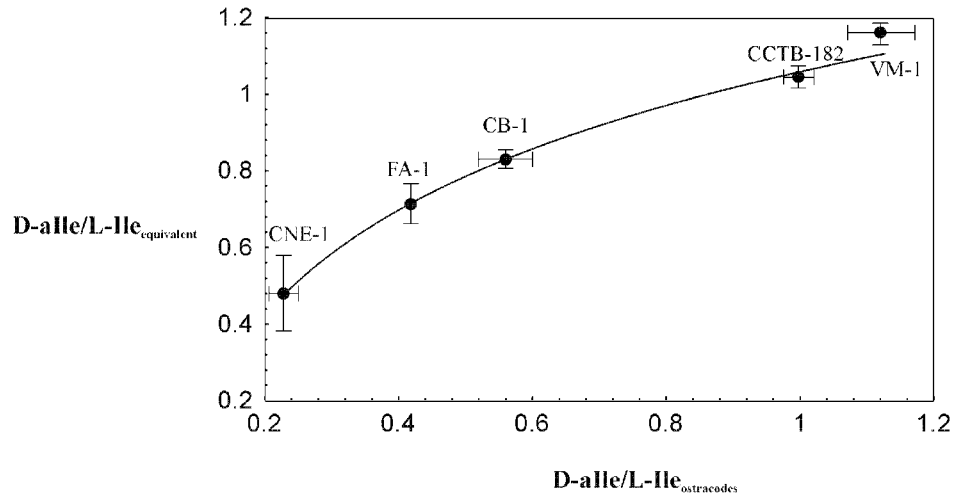

Fig. 2. Mean D-alloisoleucine/L-isoleucine values logarithmic regression plot of ostracodes and gastropods from different paleontological sites of the Cúllar-Baza Basin. Venta Micena-1 (VM-1), Cortes de Baza-182 (CTB-182), Cúllar-Baza-1 (CB-1), Fuente Amarga-1 (FA-1) and Cerro del Negro-1 (CNE-1).

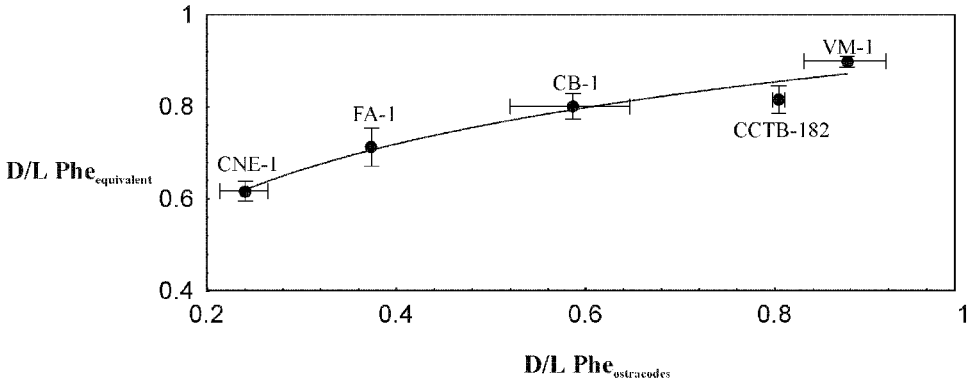

Fig. 3. Mean phenylalanine D/L values logarithmic regression plot of ostracodes and gastropods from different paleontological sites of the Cúllar-Baza Basin. Venta Micena-1 (VM-1), Cortes de Baza-182 (CTB-182), Cúllar-Baza-1 (CB-1), Fuente Amarga-1 (FA-1) and Cerro del Negro-1 (CNE-1).

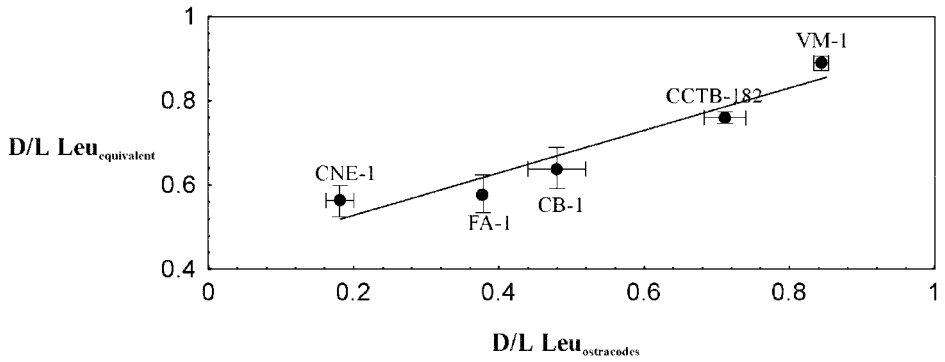

Fig. 4. Mean leucine D/L values linear regression plot of ostracodes and gastropods from different paleontological sites of the Cúllar-Baza Basin. Venta Micena-1 (VM-1), Cortes de Baza-182 (CTB-182), Cúllar-Baza-1 (CB-1), Fuente Amarga-1 (FA-1) and Cerro del Negro-1 (CNE-1).

(2000) who analyzed the reliability of diverse amino acids from different genera of marine pelecypoda in old samples, noting that isoleucine and leucine results are the most reliable. Some authors, e.g. Hearty et al. (1986) calculated for marine pelecypoda shells from Mallorca (Spain) the "equivalent ratios" only by the division of the D/L values of the "standard genus" by those in the other genus using only a single sample, applying this relationship directly as a non-proportionality factor. It is obvious that the correlation analysis using more samples than in the present work will provide more accurate results.

The leucine, phenylalanine and isoleucine D/L ratios of the gastropods and ostracodes, which are the only ones that are well correlated, were plotted in comparative histogram plots; it can be seen that in the modern samples these amino acids racemize faster in gastropods than in ostracodes (Fig. 5). However, ostracode D/L ratios become closer to those of gastropods with age, being similar in old samples. This is another reason why we think that for D/L ratios below those for the ostracodes and gastropods in the Cortijo del Negro-1 sample, these relationships cannot be applied, especially for the leucine linear approach.

Our tentative interpretation is that the differences in the racemization rates of ostracodes and gastropods are mainly due to the taxa-effect, that is the different geochemical composition of their shells since they belong to different groups of fossils. While ostracode valves are mainly made of low-magnesium-calcite (Sohn, 1958; Cadot and Kaesler, 1977; Bordegat, 1979, 1985), gastropod shells are made of aragonite (Moore 1969). The lower stability of aragonite, which changes to calcite, can explain why gastropods initially racemize faster. This could explain why the differences in the racemization rates are in some cases described by linear regression and in others by a logarithmic approach.

The intra-genera effect (Murray-Wallace, 1995) has also to be taken into account, although according to our experience the differences in D/L ratios tend to diminish with time. The relationships have been calculated using gastropods and ostracodes from different genera and in spite of the small differences that have been reported, some error has to be assumed.

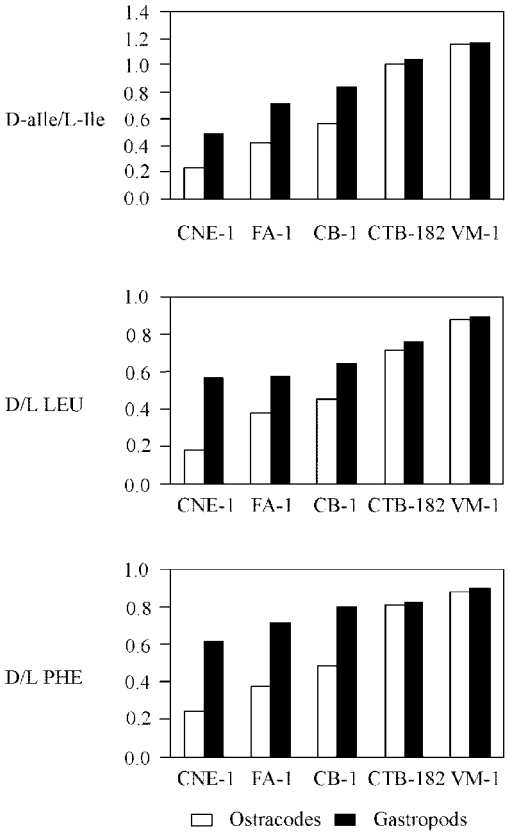

Fig. 5. Comparative evolution of leucine, phenylalanine and isoleucine D/L ratios between gastropods and ostracodes with age from the sites.

Table 3
Amino acid racemization ratios of ostracodes from samples FRA-1 and NOR-1 and comparison of the ages obtained using (1) the amino acid racemization method and (2) previous dating

Sample	n	D-aIle/L-Ile		D/L Leu		D/L Phe		Age 1 (ka)	Age 2 (ka)
		Mean	S.D.	Mean	S.D.	Mean	S.D.		
FRA-1	3	0.725	0.046	0.570	0.061	–	–	746±46	ca. 780
NOR-1	5	0.504	0.055	0.318	0.048	0.429	0.085	445±30	ca. 419–412

n, Number of analysis; S.D., standard deviation.

With these correlations, equivalent ratios can be obtained from the leucine, phenylalanine and isoleucine D/L and D-aIle/L-Ile ratios analyzed either for ostracodes or in gastropods.

In order to check the relability of these equations we took some ostracode samples (*Cyprideis torosa*) from two previously-dated horizons from the Cúllar-Baza Basin. According to the magnetostratigraphic studies of the geological record of this Basin (Oms et al., 1994; Ortiz, 2000), two important palaeomagnetic events, the Matuyama/Brunhes boundary (780 ka; Cande and Kent, 1995) and a short reverse polarity event which can be correlated to either Emperor or Lake Biwa III excursions, dated as ca. 419 ka and ca. 412 ka (Cande and Kent, 1995), have been reported. These horizons were named FRA-1 (latitude: 37°38′57′; longitude: 2°44′50″; elevation: 782 m) and NOR-1 (latitude: 37°47′4″; longitude: 2°29′0″; elevation: = 1004 m) respectively. D/L ratios are in Table 3. Unfortunately, for FRA-1 samples, the phenylalanine peaks were not satisfactorially isolated in the chromatogram.

In order to obtain the ages of the horizons we first calculated the equivalent D/L ratios using the equations obtained in this work; then, they were introduced in the age calculaton algorithms.

Isoleucine :

$$\sqrt{t} = 1.0484 + 14.088\ \mathrm{Ln}\left[\frac{\mathrm{D-alle/L-Ile}}{0.565 - \dfrac{\mathrm{D/L}}{1 + \mathrm{D-alle/L-Ile}}}\right]$$

Leucine:

$$\sqrt{t} = 0.41668 + 13.857\,\mathrm{Ln}\left[\frac{1 + \mathrm{D/L}}{1 - \mathrm{D/L}}\right]$$

Phenylalanine:

$$\sqrt{t} = 0.79019 + 11.110\mathrm{Ln}\left[\frac{1 + \mathrm{D/L}}{1 - \mathrm{D/L}}\right]$$

The ages resulting after calculating the D/L equivalent ratios of ostracodes from these three localities and using the age calculation algorithms are shown in Table 3, where it can be seen that the results are reliable.

4. Conclusions

The cross-calibration of the racemization and epimerization rates of leucine and isoleucine, respectively, between ostracodes and gastropods over the Middle and Lower Pleistocene in southern Spain have been calculated, resulting in:

$$\mathrm{D\text{-}aIle/L\text{-}Ile_{equivalent}} = 1.0716 + 0.40618 \times (\mathrm{D\text{-}alle/L\text{-}ile_{ostracodes}})$$

$$\mathrm{D/L\ LEU_{equivalent}} = 0.42581 + 0.50249 \times \mathrm{D/L\ LEU_{ostracodes}}$$

$$\mathrm{D/L\ PHE_{equivalent}} = 0.89643 + 0.19391 \times (\mathrm{D/L\ PHE_{ostracodes}})$$

With these three relationships it should be possible to either estimate the aminostratigraphy of south and central Spain, or to calculate accurately the ages of ostracode samples for a time range between ca. 1 My and 200 ka because of the special characteristics of this taxa (excellent preservation of amino acids and abundance that makes the variance smaller) by using the age calculation algorithms for gastropod D/L ratios defined by Torres et al. (1997) and Ortiz (2000). For values lower than those in the ostracode and gastropod D/L ratios in the Cortijo del Negro-1 sample, the cross-calibration equations cannot be satisfactorily applied, especially for the leucine linear approach.

The application of these equations to samples from central and south Spain, located in the Mediterranean climatic zone of the Iberian Peninsula, is based on the basis that a similar thermal history can be inferred for both areas from their identical CMAT (Current Mean Annual Temperature); cf. Torres et al., 1994, 1997). It

has been observed that the amino acids in ostracodes racemize more slowly than those in gastropods in the early stages but the D/L ratios become similar in old samples.

Acknowledgements

Funding was obtained through the project "Evolución Paleoclimática de la Mitad Sur de la Península Ibérica" of ENRESA (National Company for Radioactive Waste Management). Robert Rhew from Scripps Institution of Oceanography made helpful comments on an earlier draft. We are indebted to Dr. Veronika Meyer of the University of Bern who helped in the setting up of our laboratory. Dr. Glenn Goodfriend from the Carnegie Institution in Washington sent us the analysis protocol and GC program. The Biomolecular Stratigraphy Laboratory has been partially funded by ENRESA. We want to thank Professor Michael Engel and Professor Mark Teece for reviewing the manuscript.

Associate Editor—**J.R. Maxwell**

References

Bada, J.L., Schroeder, R.A., 1972. Racemization of isoleucine in calcareous marine sediments: kinetics and mechanism. Earth and Planetary Science Letters 15, 1–11.

Bordegat, A.M., 1979. Teneurs relative en phosphore, potassium et aluminium dans le caparace d'ostracodes actuels. Intérêt écologique (analyse à la microsonde électronique). In: Krstic, N. (Ed.), Taxonomy, Biostratigraphy and Distribution of Ostracodes. Proceedings of the 7th International Symposium on Ostracodes. Serbian Geological Society, Belgrade, pp. 261–264.

Bordegat, A.M., 1985. Composition chimique des carapaces d'ostracodes. Paramètres du milieu de vie. Atlas des ostracodes de France, Bulletin des Centres de Recherches et Exploration-Production Elf-Aquitaine. Pau 9, 379–386.

Cadot, H.M., Kaesler, R.L., 1977. Magnesium content of calcite in caparaces of benthic marine Ostracoda. The University of Kansas Paleontological Contributions 87, 1–23.

Cande, S.C., Kent, D.V., 1995. Revised calibration of the geomagnetic polarity time scale for the late Cretaceous and Cenozoic. Journal of Geophysical Research 100, 6093–6095.

Goodfriend, G.A., 1991. Patterns of racemization and epimerization of amino acids in land snail shells over the course of the Holocene. Geochimica et Cosmochimica Acta 55, 293–302.

Goodfriend, G.A., Meyer, V., 1991. A Comparative study of the kinetics of amino acid racemization/epimerization in fossil and modern mollusc shells. Geochimica et Cosmochimica Acta 55, 3355–3367.

Goodfriend, G.A., Stanley, D.J., 1996. Reworking and discontinuities in Holocene sedimentation in the Nile Delta: documentation from amino acid racemization and stable isotopes in mollusk shells. Marine Geology 129, 271–283.

Hearty, P.J., 1987. New data on the Pleistocene of Mallorca. Quaternary Science Reviews 6, 245–257.

Hearty, P.J., Miller, G.H., Stearns, C.E., Szabo, B.J., 1986. Aminostratigraphy of Quaternary Shorelines around the Mediterranean basin. Geological Society American Bulletin 97, 850–858.

Kaufman, D.S., 2000. Amino acid racemization in ostracodes. In: Goodfriend, G.A., Collins, M.J., Fogel, M.L., Macko, S.A., Wehmiller, J.F. (Eds.), Perspectives in Amino Acids and Protein Geochemistry. Oxford University Press, New York, pp. 145–160.

King, K., Neville, C., 1977. Isoleucine epimerization for dating marine sediments: importance of analyzing monospecific foraminiferal samples. Science 195, 1333–1335.

Kriausakul, N., Mitterer, R.M., 1980. Comparison of isoleucine epimerization in a model dipeptide and fossil protein. Geochimica et Cosmochimica Acta 44, 753–758.

Masters, P., Bada, J.L., 1977. Racemization of isoleucine in fossil molluscs from Indian middens and interglacial terraces in Southern California. Earth and Planetary Science Letters 37, 173–183.

Martínez Navarro, B., 1992. Revisión sistemática de la fauna de macromamíferos del yacimiento de Venta Micena (Orce, Granada, España). In: Gibert, J. (Ed.), Presencia humana en el Pleistoceno inferior de Granada y Murcia. Proyecto Orce-Cueva Victoria (1988–1992). Museo de Prehistoria Josep Gibert, Gràfiques Castellar, Barcelona, pp. 21–85.

McCoy, W.D., 1988. Amino acid racemization in fossil nonmarine ostracod shells: a potential tool for the study of Quaternary stratigraphy, chronology and palaeotemperature. In: De Deckker, P., Colin, J.P., Peypouquet, J.P. (Eds.), Ostracoda in the Earth Sciences. Eselvier, Amsterdam & New York, pp. 201–218.

Miller, G.H., Sejrup, H.P., Mangerud, J., Andersen, B.G., 1983. Amino Acid ratios in Quaternary mollucs and foraminifera from western Norway: correlation, geochronology and paleotemperature estimates. Boreas 12, 107–124.

Moore, R.C. (Ed.), 1969. Treatise on invertebrate palaeontology Part I. Mollusca 1 (Principles, gastropods). Geological Society of America, University of Kansas Press, Lawrence, KS.

Murray-Wallace, C.V., 1995. Aminostratigraphy of Quaternary coastal sequences in Southern Australia—an overview. Quaternary International 26, 69–86.

Oms, O., Garcés, M., Parés, J.M., Agustí, J., Anadón, P., Juliá, R., 1994. Magnetostratigraphic characterization of a thick Lower Pleistocene lacustrine sequence from the Baza Basin (Betic Chain, Southern Spain). Physics of the Earth and Planetary Interiors 85, 173–180.

Ortiz, J.E., 2000. Evolución paleoclimática durante el Pleistoceno de la mitad sur de la Península Ibérica mediante el estudio paleontológico y geoquímico de ostrácodos de la cuenca de Cúllar-Baza (Granada, España). PhD thesis, Politechnical University of Madrid.

Ortiz, J.E., Torres, T., Llamas, J.F., Canoira, L., García-Alonso, P., García de la Morena, M.A., Lucini, M., 2000. Dataciones de algunos yacimientos paleontológicos de la cuenca de Guadix-Baza (sector de Cúllar-Baza, Granada, España) y primera estimación de edad de la apertura de la cuenca mediante el método de racemización de aminoácidos. Geogaceta 28, 109–112.

Oviatt, C.G., Thompson, R.S., Kaufman, D.S., Bright, J.,

Forester, R.M., 1999. Reinterpretation of the Burmester core, Bonneville Basin, Utah. Quaternary Research 52, 180–184.

Sesé, C., 1994. Paleoclimatical interpretation of the Quaternary small mammals of Spain. Geobios 27 (6), 753–767.

Sohn, I.G., 1958. Chemical constituents of ostracodes: some applications to paleontology and paleoecology. Journal of Paleontology 32, 730–736.

Torres, T., Canoira, L., Cobo, R., García, P., García Cortés, A., Juliá, R., Llamas, J., Hoyos, M., Meyer, V., 1994. Aminoestratigrafía y aminozonación de los travertinos fluviales de Priego (Cuenca, España Central). Geogaceta 17, 102–105.

Torres, T., Canoira, L., Coello, J., García-Alonso, P., Llamas, J., 1995. Aminoestratigrafía y Geocronología por análisis de racemizaciín de aminoácidos de muestras de gasterópodos y lamelibranquios de la Cuenca de Cúllar-Baza (Granada). MAGNA Project Report (Ingemisa).

Torres, T., Llamas, J., Canoira, L., García-Alonso, P., García-Cortés, A., Mansilla, H., 1997. Amino acid chronology of the Lower Pleistocene deposits of Venta Micena (Orce, Granada, Andalusia, Spain). Organic Geochemistry 26, 85–97.

Torres, T, Llamas, J., Canoira, L., Coello, F.J., García-Alonso, P., Ortiz, J.E., 2000. Aminostratigraphy of two Pleistocene marine sequences from the Mediterranean Coast of Spain: Cabo de Huertas (Alicante) and Garrucha (Almería). In: Goodfriend, G.A., Collins, M.J., Fogel, M.L., Macko, S.A., Wehmiller, J.F. (Eds.), Perspectives in Amino Acids and Protein Geochemistry. Oxford University Press, New York, pp. 263–278.

Wehmiller, J.F., Hare, P.E., 1971. Racemization of amino acids in marine sediments. Science 173, 907–911.